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Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(54) Title: DIETARY SUPPLEMENT

(57) Abstract: The present invention concerns a method for the manufacture of a composition of matter for dietary intake, such as a food or feed product or food supplement, for strengthening the antioxidative defence system of a subject, such as a human. The method is characterized in that to the composition, folic acid or folate

Dietary supplement

The present invention relates to a novel use of folic acid or folate, or of a functionally equivalent substance having the biochemical activity of folic acid or folate, such as folinic acid, as a dietary supplement in or for fortification of compositions of matter, such as products for ingestion or dietary intake, for example food and feedstuffs.

Although low serum folate concentration has been established as a new risk factor for cardiovascular diseases (CVD), the mechanism underlying the risk-lowering effect is unclear. Folate is an important substrate in the remethylation of homocysteine (tHcy) back to methionine and it is supposed that folic acid could lower the risk of CVD through reducing plasma tHcy concentrations[1].

The human serum paraoxonase (PON) is an antioxidative enzyme in high density lipoproteins (HDL), which eliminates lipid soluble radicals in the circulation and protects against coronary disease[2-4]. There is epidemiologic evidence showing that persons with elevated HDL cholesterol levels are at reduced risk of coronary heart disease (CHD). HDL has been shown to possess antioxidative properties, which might conceivably contribute to the protection by HDL against CHD. Paraoxonase/arylesterase has been suggested to account for an important part of the antioxidative property of HDL[5]. A lowered PON activity has been reported also in patients with myocardial infarction (MI)[2-3].

The present invention is based on the finding that there is a positive correlation between circulating folate or dietary folate intake and enhanced serum PON activity. Enhanced serum PON activity improves the endogenous antioxidative capacity and defences of a subject, *e.g.* a human, thus reducing the risk *e.g.* for CHD, cancer, type II diabetes and cataract, and is also associated with the process of aging.

In its broadest sense the invention is directed to a method for enhancing the endogenous antioxidative defences of the body by dietary folic acid or folate supplementation. The term "folate" refers to the salts and esters of folic acid.

Acceptable salts include i.e. the alkali metal salts, such as the sodium salt and the methylglucamine salt. Esters of folic acid can be prepared in a manner known to the person skilled in the art.

The object of the invention is thus a method for the manufacture of a composition of matter for dietary intake with antioxidative defences enhancing effect, according to which method, in the said composition, folic acid or folate or a functionally equivalent substance is used as the active or effective agent.

According to a second aspect, the object of the invention is the use of folic acid or folate or a functionally equivalent substance as a supplement or additive for the manufacture of a composition of matter for dietary intake with antioxidative defences enhancing effect.

According to a third aspect, the object of the invention is a method for enhancing the antioxidative defences in a subject comprising administering, *e.g.* orally or parenterally, to said subject, an effective amount of folic acid or folate or a functionally equivalent substance.

A functionally equivalent substance of folic acid or folate is intended to mean a substance that has the biochemical activity of folic acid, for example folinic acid or a salt or ester of folinic acid.

The term "subject" means here a mammal, such as a human, or an animal, especially livestock or farm animals.

The term "effective amount" means an amount of the active agent which after administration is sufficient to enhance the serum paraoxonase activity and thus the antioxidative defences in the subject. In the following, the terms folic acid and folate can be used interchangeably.

The composition of matter can be any dietary, typically orally administered composition, preparation or product, such as a food or feed product, food supplement, a drug preparation, or a raw material for such a product.

The product to be used as the composition of matter to be supplemented or fortified by adding thereto folic acid or folate or a functionally equivalent its derivative, can in principle be any type of food or feed product. According to an exemplary embodiment of the invention, it is contemplated to add folic acid or a functionally equivalent derivative thereof to grain, and to use such supplemented grain in grain based foods or feeds, or to add it as a supplement to such foods or feeds, such as to bakery products, cereals, snack foods, beverages *etc.* However, it is possible to include folic acid or its derivative in any type of food or feed product, in which it can be properly included and distributed, such as processed foods, for example meat products, such as sausages, or also other ready made foods. It is also possible to fortify dairy products, such as milk, cheese, butter and youghurt, as well as other beverages, such as fruit drinks and juices, with the dietary supplement according to the invention.

A dietary supplement can also be in the form of tablets, capsules, lozenges, granules, syrups, solutions, suspensions for oral administration, wherein the folic acid or folate is suitably included together with a carrier or filler substance, such as lactose, silica, glucose, starch, glycerol, diluents and solvents. Such dosage forms may include conventional additives such as glidants, stabilizer, colouring agents, preservatives, taste improving agents as well as a matrix to slow down absorption such as methyl cellulose or microcrystalline cellulose with colloidal anhydrous silica. The preparation of such supplements in dosage form is well known to the person skilled in the art.

The amount of folic acid or the functionally equivalent substance to be included in a composition of matter for dietary intake, can vary broadly depending on the type of product to be supplemented or fortified, as well as on its intake frequency and intake levels. Such amounts can easily be determined by the person skilled in the

art, to provide for and secure a suitable daily total intake of the dietary supplement. A typical amount for intake would be for example 15 to 1200 μg of folate per day for a subject of normal size, such as a human subject of appr. 80 kg, or a daily intake ranging from appr. 0.2 to 15 μg per kg body weight. This amount can be administered for example in bread, such as in 100 g of bread constituting a suitable single portion.

Experimental section

Serum PON enzyme activity was analyzed based on its capacity to hydrolyze paraoxon, and erythrocyte folate levels were measured by RIA. First the association between erythrocyte folate levels and serum PON activity in a population-based sample of 155 men aged 53-71 years examined in 1998-99 as a part of the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) was examined. The correlation coefficient for the association between serum PON activity and erythrocyte folate levels was 0.25 (P = 0.002). The unadjusted mean (95 % confidence interval (CI)) serum PON activity was 26.3 % lower (63.2, 47.5 to 79.0 nmol/mL/min) in the lowest fifth of erythrocyte folate concentration than in the highest fifth (85.7, 63.2 to 108.2 nmol/mL/min).

To confirm the observational finding in an experiment, the effect of oral folate supplementation on PON activity in a placebo-controlled double-blind folic acid supplementation trial was studied. In this study 39 healthy voluntary men aged 19-36 years were randomized to receive either 0.9 mg folate or placebo daily for 8 weeks. The correlation coefficient for the association between changes in erythrocyte folate concentration and PON activity during the study period was 0.36 (P = 0.025). The mean serum PON activity increased by 4.0 % (2.43 nmol/mL/min) in the folic acid group and decreased by 3.6 % (-2.87 nmol/mL/min) in the placebo group (P = 0.015 for the difference between groups). The correlation coefficient for the association between changes in erythrocyte folate concentration and plasma total homocysteine (tHcy) concentration was -0.62 ($P < 0.001$), and that for changes in plasma tHcy concentration and serum PON enzyme activity -0.25 (P = 0.134). In

a linear regression model strongest predictors of change in serum PON activity were the change in erythrocyte folate concentration (standardized coefficient 0.41, P = 0.010), and age (0.27, P = 0.091) (adjusted R Square for the model 0.151, P = 0.020) .

In summary, the experimental findings indicate that folate supplementation and blood folate concentration affect serum PON activity in humans.

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Claims

1. Method for the manufacture of a composition of matter for dietary intake with antioxidative defences enhancing effect, characterized in that folic acid, folate or a substance which is functionally equivalent thereto is used as the active agent in the said composition.

2. The method according to claim 1, characterized in that the composition of matter is a food or feed product, a food supplement, a drug preparation, or a raw material therefor.

3. The method according to claim 1 or 2, characterized in that to the composition of matter an amount of active agent is added which provides for a daily dietary intake of 0.2 to 15 μg of folic acid or folate, per kg of body weight of a subject to which the composition is administered.

4. The method according to claim 1 or 2, characterized in that the composition of matter provides a daily intake of 15 to 1200 μg of folic acid or folate, for example in a bakery product.

5. Use of folic acid or folate or a substance which is functionally equivalent thereto as a supplement or additive for the manufacture of a composition of matter for dietary intake with antioxidative defences enhancing effect .

6. The use according to claim 5 wherein the composition of matter is a food or feed product, a food supplement, a drug preparation, or a raw material therefor.

7. A method for enhancing the the antioxidative defence system in a subject comprising administering to said subject an effective amount of folic acid or folate or a substance which is functionally equivalent thereto.

8. The method according to claim 7 comprising administering to the said subject 0.2 to 15 μg per kg of body weight per day of folic acid or folate or a functionally equivalent substance.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 00/00899

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A23L 1/302, A61K 31/519, A61P 9/00, A61P 39/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A23L, A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC, WPI, CAPLUS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, file CAPLUS, CAPLUS accession no. 2000:89946, Document no. 133:30090, Hsu, Yu-Chin et al: "Effects of marginal folate deficiency on folate status, antioxidant capacity and lipid peroxidation in F344 rat livers"; & Zhonghua Minguo Yingyang Xuehui Zazhi (1999), 24(3), 228-240	1-8
A	US 6121249 A (DONALD L. WEISSMAN ET AL), 19 Sept 2000 (19.09.00)	1-8
A	EP 0595005 A1 (VESTA MEDICINES (PROPRIETARY) LIMITED), 4 May 1994 (04.05.94)	1-8

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 January 2001	30 -01- 2001
Name and mailing address of the ISA	Authorized officer

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 00/00899

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9714422 A1 (HERBERT, VICTOR, D.), 24 April 1997 (24.04.97)	1-8
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A	DE 29808384 U1 (ECKES-GRANINI GMBH & CO. KG), 17 Sept 1998 (17.09.98)	1-8
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A	STN International, file CAPLUS, CAPLUS accession no. 1999:158555, Document no. 130:310055, Pfohl, Martin et al: "Paraoxonase 192 Gln/Arg gene polymorphism, coronary artery disease, and myocardial infarction in type 2 diabetes"; & Diabetes (1999), 48(3), 623-627	1,5,7
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI00/00899

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 7, 8
because they relate to subject matter not required to be searched by this Authority, namely:
see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI00/00899

Claims 7, 8 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/FI 00/00899

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6121249 A	19/09/00	NONE	
EP 0595005 A1	04/05/94	CA 2105177 A	15/03/94
		CN 1087517 A	08/06/94
		JP 6192105 A	12/07/94
		ZA 9306723 A	14/08/95
WO 9714422 A1	24/04/97	CA 2234954 A	24/04/97
		EP 0859619 A	26/08/98
		US 5932624 A	03/08/99
DE 29808384 U1	17/09/98	NONE	